View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Vietnam Academy of Science and Technology: Journals Online

Journal of Science and Technology **54** (2C) (2016) 479-485

INVESTIGATION OF INENZYME TREATMENTS TO ASSIST EXTRACTION OF ESSENTIAL OIL FROM THE LEAVES AND BRANCHES OF *CINNAMOMUM CASSIA* COLLECTED IN YEN BAI PROVINCE

Hoang Thi Bich[1, *], Le Tat Thanh[1], Tran Quoc Toan[1], Nguyen Huy Tung[1], Le Xuan Duy[1], Nguyen Van Tuyen Anh[1], Dinh Thi Thu Thuy[1], Tran Thi Tuyen[1], Do Trung Sy[2], Le Mai Huong[1, *], Nguyen Quyet Chien[2]

[1]*Institute of Natural Products Chemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi*

[2]*Institute of Chemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi*

[*]Email: *bichhoang.inpc@gmail.com; lehuong1258@gmail.com*

Received: 15 June 2016; Accepted for publication: 29 October 2016

ABSTRACT

In order to improve the efficiency of essential oil distillation of the leaves and branches from the cassia plant (*Cinnamomum cassia* (L.) J. Presl) growing in Yen Bai province, the effects of enzyme treatments of the plant materials before distillation were investigated using crude laccase obtained from culture medium of the fungus *Ganoderma lucidum* and Cellic Htech 2 (Novozymes, Denmark), a multienzyme system consisting of cellulase and xylanase. The results showed that enzyme treatments increased oil yield and shorten distillation time. The use of a mixture of both enzyme systems was more effective than using them separately. Under optimal conditions, an increase in oil yield of 41.7 % was achieved, while distillation time was shortened from 8 to 5 hours. The enzyme treatments did not change the qualitative composition of the essential oils. However, significant changes in the percentages of cinnamic aldehyde (69.74 % to 85.6 %) and cinnamyl acetate (17.2 % to 1.34 %) were observed.

Keywords: enzyme assisted extraction, cassia oil, *Cinnamomum cassia*, cellulase, laccase.

1. INTRODUCTION

Enzyme-assisted extraction is a modern terminology for methods using enzymes to improve the efficiency of extraction processes of valuable natural products [1]. This technique uses a specific enzyme or enzyme systems capable of breaking down various cell wall components (cellulose, hemicellulose and lignin) to facilitate better contact between the extraction solvents and target compounds. In the past, this technique has mostly been considered as a technological know-how and kept as a secret, thus hindering its general applications. As most of the basic principles of these techniques are scientifically clarified, the scope of its application has been increasingly expanded. At present, enzyme-assisted extraction is not only a

good choice for the improvement of process efficiency but also has become an important tool of green chemistry, allowing the creation of new energy- and cost-effective technologies using environmentally friendly chemicals [2, 3].

Cassia, *Cinnamomum cassia* (L.) J. Presl (Lauraceae), is an endemic plant of Vietnam, traditionally grown for its barks as a spice and for the extraction of its essential oil (cassia oil). Production areas reaching 16.000 hectares are concentrated in the provinces of Quang Nam, Quang Ngai, Quang Ninh, and Yen Bai [4]. Vietnamese cassia oil is mainly extracted from cassia leaves and branches by small producers in Yen Bai and Lao Cai provinces using traditional hydrodistillation technique. Recently a new technique learned from Chinese producers, which involves a fermentation step of the raw materials before distillation, led to an apparent improvement of the distillation yield. Recognizing the relationship of this technique with the science of enzyme-assisted extraction, in this study we investigate various aspects of using enzymes for the improvement of this distillation process. Enzyme systems under investigation were Novozymes Cellic Htec 2 (mainly effective for the degradation of cellulose and hemicellulose) and a crude mixture of laccases obtained from culture medium of the fungus *Ganoderma lucidum* (mainly effective for lignin degradation). Their effects on the degradation of the cell wall components, oil yields, distillation time and chemical composition of the essential oils were examined.

2. METHODS AND MATERIAL

2.1. Plant material

Samples of *Cinnamomum cassia* (L.) J. Presl (Lauraceae) were collected in Van Yen district, Yen Bai province. Leaves and branches were dried in the shadow and then finely ground into a powder with a size smaller than 0.1 mm, packed in polyethylene bags under vacuum, and stored in the dark at room temperature until use.

2.2. Enzymes

The crude laccase was isolated from culture medium of the fungus *Ganoderma lucidum* by procedures described by Ding *et al*. [5]. Laccase activity was determined spectrophotometrically by measuring the increase in absorbance at 420 nm, 30 °C using 1 mM ABTS (2,2'-di--azino [3-ethyl-benzothiazolin- sulphonate]) as substrate.

Cellic Htec 2, an enzyme preparation consisting of cellulase and xylanase, was purchased from Novozymes (Bagsvaerd, Denmark). Its cellulase and xylanase activities were assayed by methods described by Wood and Bhat [6] and Bailey [7].

2.3. Analytical methods

2.3.1. Extraction of essential oils

Essential oils were extracted from plant materials by hydrodistillation. Before extraction, 100 g of cassia leaf powder were subjected to a preliminary treatment by soaking in water for 24 hours (material/water 1:5, g/ml). Then, the material was steam distilled in a 2 L Clevenger-type apparatus for specified times (2 - 8 hours). The obtained essential oil was dried over anhydrous

sodium sulfate and stored in a sealed vial at 10°C in the dark prior to analysis. Oil yield is calculated based on the mass of essential oil obtained and the mass of the initial material.

2.3.2. Determination of the chemical composition of the essential oils

The chemical composition of the essential oils was determined by GC-MS method on an Agilent HP mode 7890A gas chromatograph coupled to an Agilent 5973 mass spectrometer. Data acquisition and processing were performed using Agilent MSD productivity Chemstation Rev. E-02.02. Retention indices of the oil constituents were determined on the HP5-MS column using standard C7 – C30 straight chain hydrocarbons (Aldrich Chemical Company, USA). Individual compounds in the oil were identified by comparison of their mass spectra and retention indices with those in GC-MS libraries (NIST 08, Wiley 09 and HPCH1607).

2.3.3. Treatment of cassia powder with enzymes

100 g of cassia leaves and branches powder were first pre-treated as described above, then put into a 2 L glass beaker equipped with a hot plate and a stirrer. After adjusting the medium to a pH value of 5.0, specified amounts of enzymes were added. Incubation was then performed under stirring (90–120 rpm) at 45^{o}C for 12 hours.

For analysis, 20 mL of the reaction product was taken and centrifuged at 6.000 rpm for 15 minutes. The liquid part was used for the determination of reducing sugars by the DNS method as described by Miller [8]. The solid part was thoroughly washed with distilled water, dried at 70–85^{o}C to obtain a constant weight and used for the determination of residual lignin by the denaturation method as described by Jang and Argyropoulos [9].

3. RESULTS AND DISCUSSION

3.1. The influence of the processing by enzyme to distilled performance and time oils

Crude enzyme mixture isolated from culture medium of the fungus *Ganoderma lucidum* and the commercial Cellic Htec 2 (Novozymes) were used to study the effects of enzyme treatments on oil yields, distillation time and chemical composition of the essential oils in relationship to the degradation of the cell wall components cellulose, hemicellulose, and lignin. The crude enzymes from *G. lucidum* consist mainly of lignin degrading laccases [8], while Cellic Htec 2 (Novozymes) is a mixture of cellulase and endoxylanase, which degrade cellulose and hemicellulose. Enzyme activity was assayed and presented in Table 1.

Table 1. Enzyme activities.

No	Enzymes	Assays	Substrates	Unit	Activity
1	Crude enzymes from *G. lucidum*	Laccase	ABTS	UI/ml	185
2	Cellic Htec2	Cellulase Xylanase	CMC Xylan	UI/g	2800 1500

The effects of enzyme treatments on oil yields and degradation of cell wall components are summarized in Table 2. The release of cassia oils was 7.3–41.7 % higher than that from the untreated samples. The magnitude of such an enhancement was greater by using combination of enzyme (laccase, Htec2) than single enzyme (laccase or Htec2). This increase in recovery can be

attributed to the ability of enzymes to degrade cell wall structure and depolymerize plant cell wall polysaccharides, facilitating the release of essential oil (Boulia *et al.* [10]).

Table 2. Effects of enzyme treatments on the degradation of cell wall component and oil yields.

No	Enzymes	Reducing sugars (µg/ml)	Lignin (%)	Oil yield (%)	Gain in oil yield (%)
1	No enzymes	0.0	17.43	0.69	0
2	Crude laccase from *G. lucidum*	3.12	14.21	0.74	7.3
3	Cellic Htec2	18.13	15.32	0.81	16.9
4	Crude laccase + Cellic Htec2	78.14	13.01	0.98	41.7

Increase in yield of essential oil after enzyme pre-treatment has been reported earlier for garlic cloves , celery seeds , cumin seed , black pepper and cardamom , thyme and rosemary or bay leaves *Laurus nobilis L.* [10]. The results showed that enzyme treatments significantly increased the yields of essential oils as compared with control experiments. The increase in oil yields correlates well with the increase of reducing sugar and the decrease of lignin contents of the distillation materials, which is a concrete evidence for the impact of the enzyme's activities.

As shown in Table 2, the best gain in oil yield of 41.9 % was obtained by treatment of the plant materials with a mixture of both enzyme systems. Optimal conditions were found to be as follows: concentration of crude laccase from *G. lucidum*: 70 UI/g substrate, Cellic Htec2 concentration: 1.5 % of substrate, pH 5, temperature 50 ^{o}C, stirring speed 150 rpm, duration of incubation 5 hours.

The use of this enzyme system also helped to significantly decrease distillation time (Figure 1). With enzyme treatment, after the first 2 hours, more than 50 % of the total content of essential oil has already been recovered. Maximum oil yield was obtained after 5 hours. For comparison, with traditional method without enzyme treatment the first 2 hours afforded only about 10% of the total content of essential oil. Maximum oil yield was obtained only after 7–8 hours.

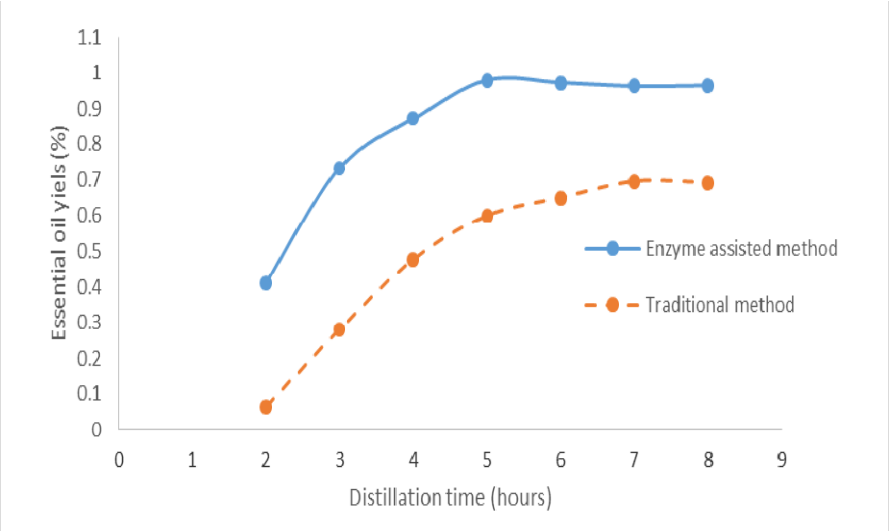

Figure 1. Effects of enzyme treatment on distillation time.

3.2. Effects of enzyme treatments on chemical composition of the essential oils

The chemical compositions of the essential oils obtained by the experiments described in Table 2 were determined by GC-MS method.

Table 3. Chemical composition of the essential oils obtained by the 4 experiments.

No	Compounds	Content (%)			
		Control	Laccase	Htec2	Laccase & Htec2
1	Benzaldehyde	1.62	1.93	1.77	0.69
2	Salicylaldehyde	0.45	0.51	0.87	0.61
3	Unknown				0.64
4	Benzenepropanal	1.59	1.64	1.14	0.78
5	Benzofuran	5.9	4.52	1.64	2.44
6	*cis*-Cinnamaldehyde	0.75	0.78	0.4	1.23
7	Phenylethyl acetate	-		0.11	-
8	*trans*-Cinnamaldehyde	69.74	68.96	70.54	85.6
9	Unknown	-	-	-	0.16
10	Cinnamyl alcohol	0.57	0.58	0.17	0.29
11	α-Copaene	0.68	0.8	0.32	0.66
12	Caryophylene	0.26	0.3	-	0.26
13	Cinnamyl acetate	17.2	15.58	21.02	1.34
14	Coumarin	0.88	0.84	0.65	1.78
15	Caryophyllene	0.13	0.17	-	0.26
16	γ-Muurolene	0.29	0.35	0.17	0.47
17	Viridiflorene	-	0.28	-	0.16
18	α-Muurolene	-	0.18	-	0.2
19	β-Bisabolene		-	-	0.11
20	γ-Cadinene	0.17	0.21	-	0.3
21	δ-Cadinene	0.56	0.73	0.12	0.63
22	*cis*-Calamenene	-	0.11		0.21
23	*p*-Methoxycinnamaldehyde	-	-	-	0.23
24	γ-Bisabolene	0.19	0.23	-	-
25	Nerolidol	0.31	0.34	0.22	0.37
26	Caryophyllene oxide	0.12	0.13	-	0.14
27	Bisabolol	-	-	-	0.13
	Total	99.41	99.29	99.14	99.81

The results presented in Table 3 showed that enzyme treatments did not change their basic characters as compared with control. All oil samples contained cinnamic aldehyde as the major component and possessed the same number of other important components. However, enzyme treatments caused changes in the percentages of some components. Especially, experiment No. 4 using a mixture of both enzyme systems brought about the most obvious changes. In this experiment, the cinnamic aldehyde content increased from 69.74 % to 85.60 %, while the cinnamyl acetate content decreased from 17.20 % to 1.34 %, suggesting a close relationship between these variations. Other components with significant changes were methoxycinnamic aldehyde (from 0 % to 0.23 %), coumarin (from 0.88 % to 1.78 %). Generally, an increase in cinnamic aldehyde and methoxycinnamaldehyde contents improves the value of cassia oils, while an increase in coumarin content is unwanted.

4. CONCLUSION

The above results showed that treatments of cassia leaves and branches powder with enzyme systems containing laccase, Htec2 (cellulase and xylanase) before distillation significantly increased oil yields and shorten distillation times. The use of a mixture of enzymes is more efficient than using them separately. Under optimal conditions, an increase in cassia oil yield of 41.7 % was achieved, while distillation time was shortened from 8 to 5 hours. The enzyme treatments did not change the qualitative composition of the essential oils as compared with control. However, significant changes in the percentages of cinnamic aldehyde (69.74 % up to 85.6 %) and cinnamyl acetate (17.2 % down to 1.34 %) were observed. Other minor changes of concern were found for methoxycinnamic aldehyde and coumarin. Enzyme application result in extraction of natural bioactive compounds from plant showed that this application is feasible in industry. From this result, we are carrying out with some other plants such as extraction of essential oil from *Aquilaria crassna*, *Cinnamomum camphora., Fokienia hodginsii*...

Acknowledgements. This work has been completed with the support of the project "Research chemical component, investigate and assess the quality of essential oil of frankincense currently being manufactured in Vietnam". Code: VAST 04.

REFERENCES

1. Puri M., Sharma D. and Barrow C. J. - Enzyme assisted extraction of bioactives from plants, Trends in Biotechnology **30** (1) (2012) 37-44.
2. Chemat F. and Abert V. M. - Alternative Solvents for Natural Products Extraction, Green Chemistry and Sustainable Technology, Springer Verlag, 2014, pp. 324.
3. Chemat F., Strube J. - Green Extraction of Natural Products: Theory and Practice, John Wiley & Sons, 2015, pp. 384.
4. La Dinh Moi, Luu D. C., Tran M. H., Nguyen T. T., Nguyen T. P. T., Tran H. T., Ninh K. B. - Essential–oil plant recouces in Vietnam, Vol. 1, Agriculture Publishing House, Hanoi, 2001, tr. 198 -206.
5. Ding Z., Peng L., Chen Y., Zhang L., Gu Z., Shi G. and Zhang K. - Production and characterization of thermostable laccase from the mushroom, *Ganoderma lucidum*, using submerged fermentation, African Journal of Microbiology Research **6** (6) (2012) 1147-1157.
6. Wood T. M. and Bhat K. M. - Methods for measuring cellulose activities, Methods in Enzymology **160** (1988) 87-112.
7. Bailey M. J., Biely P. and Kaisa P. - Interlaboratory testing of methods for assay of xylanase activity, Journal of Biotechnology **23** (1992) 257-270.
8. Miler G. L. - Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. **3** (1959) 426-428.
9. Jiang Z. H. and Argyropoulos D. S. - Isolation and Characterization of Residual Lignins in Kraft Pulps, Journal of Pulp and Paper Science **25** (1) (1999) 25-29.
10. Boulila A., Hassen I., Haouari L., Mejri F., Amor I. B. - Enzyme-assisted extraction of bioactive compounds from bay leaves (*Laurus nobilis* L.), Industrial Crops and Products **74** (2015) 485-493.

TÓM TẮT

NGHIÊN CỨU SỬ DỤNG HỖN HỢP ENZYME HỖ TRỢ CHIẾT TÁCH TINH DẦU TỪ LÁ VÀ CÀNH QUẾ YÊN BÁI (*CINNAMOMUM CASSIA*)

Hoàng Thị Bích[1], Lê Tất Thành[1], Trần Quốc Toàn[1], Nguyễn Huy Tùng[1], Lê Xuân Duy[1], Nguyễn Văn Tuyến Anh[1], Đinh Thị Thu Thủy[1], Trần Thị Tuyến[1], Đỗ Trung Sỹ[2], Lê Mai Hương[1], Nguyễn Quyết Chiến[2]

[1]*Viện Hóa học các Hợp chất thiên nhiên, Viện Hàn lâm KH&CNVN, 18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội*

[2]*Viện Hóa học, Viện Hàn lâm KH&CNVN, 18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội*

[*]Email: *bichhoang.inpc@gmail.com; lehuong1258@gmail.com*

Nhằm nâng cao hiệu quả chưng cất tinh dầu từ lá và cành quế Yên Bái (*Cinnamomum cassia* (L.) J. Presl), ba phương án xử lí nguyên liệu sử dụng riêng rẽ và kết hợp hai hệ enzyme là laccase thô tách từ canh trường nuôi cấy nấm Linh chi (*G. Lucidum*) và Cellic Htec2 của hãng Novozymes A/S đã được khảo sát và so sánh với cách xử lí truyền thống không sử dụng enzyme. Kết quả cho thấy việc xử lí nguyên liệu có sự hỗ trợ của các enzyme đã làm tăng hiệu suất thu hồi và rút ngắn thời gian chưng cất. Việc sử dụng hỗn hợp của cả hai hệ enzyme cho hiệu quả cao hơn việc sử dụng riêng rẽ. Ở điều kiện tối ưu, hiệu suất cất tinh dầu đã tăng lên hơn 40 % so với phương pháp xử lí truyền thống, thời gian chưng cất để đạt hiệu suất tối đa cũng được rút ngắn từ 8 giờ xuống còn 5 giờ. Thành phần hóa học của tinh dầu không có thay đổi cơ bản về chất, nhưng có một số thay đổi về lượng. Hàm lượng cinnamic aldehyde tăng từ 69,74 % lên 85,6 %, cinnamyl acetate giảm từ 17,2 % xuống 1,34 %.

Từ khóa: chiết xuất có enzyme hỗ trợ, tinh dầu quế, *Cinnamomum cassia*, cellulase, laccase.